INTRODUCTION

The eLUCidate iNOS reporter cell line is a stably transfected RAW 264.7 cell line which expresses Renilla luciferase reporter gene under the transcriptional control of the iNOS promoter. Inducible nitric oxide synthase (iNOS) is an inducible enzyme that catalyzes the production of nitric oxide (NO) from L-arginine. NO is one of the smallest signaling molecules that can diffuse into the cell and is involved in various physiological functions, pathogenesis of septic shock, many diseases associated with autoimmunity and tumorigenesis. iNOS gene is generally known to be induced by various proinflammatory cytokines and pathogen-associated molecular patterns such as TLR ligands. The eLUCidate iNOS reporter cell line is tested by inducing with various toll-like receptor (TLR) ligands and phorbol 12-myristate 13-acetate and provides an effective way to monitor or screen for activators/inhibitors of the iNOS signaling pathway.

MATERIALS AND METHODS

1. General Culture Conditions

Cells should be grown at 37°C with 5% CO₂ using Growth Medium (DMEM plus 10% FBS and 1% Pen/Strep). Where needed, add 3 μg/ml Puromycin.

2. Thawing and Growing Cells

2.1 Aliquot 10 ml of Growth Medium to a 15 ml sterile tube.

2.2 Remove cells from storage and quickly thaw in a 37°C water bath.

2.3 Transfer cells to the tube in 2.1.

2.4 Spin cells down at 1,000 rpm for 10 minutes.

2.5 Resuspend cells in pre-warmed Growth Medium.

2.6 Transfer cells to a T25 flask (or equivalent) and culture at 37°C in a CO₂ incubator.

2.7 At first passage, transfer cells into Growth Medium with Puromycin.

2.8 Split cells when they reach about 80-90% confluence.

3. Cell Passaging

3.1 Detach cells using Detachin™ Cell Detachment Solution (Cat #: T100100).

3.2 Add 10 ml Growth Medium with Puromycin to cells and transfer to a sterile 50 ml centrifuge tube.

3.3 Pellet cells by centrifuging at 1,000 rpm for 10 min.

3.4 Resuspend cells in Growth Medium with Puromycin to achieve 1:10 or 1:20 dilution ratio, and plate as needed.

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